# **PCT**

REC'D 18 AUG 2004 **WIPO** PCT

# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference FOR FURTHER ACTION See Form PCT/IPEA/416 |   | PCT/IPEA/416  |  |  |  |  |  |  |
|--|---|---|--|--|--|--|--|--|
| PU0242-PCT   |   |   |  |  |  |  |  |  |
| International application No.  | International filing date (day/month/year)  | Priority date (day/month/year)                          |  |  |  |  |  |  |
| PCT/SE 2003/001127   | 26.06.2003  | 28.06.2002  |  |  |  |  |  |  |
| International Patent Classification (IPC) or national classification and IPC   |   |   |  |  |  |  |  |  |
| C12N 15/10, C07H 1/06, C07H 1/08   |   |   |  |  |  |  |  |  |
| ·  |   |   |  |  |  |  |  |  |
| Applicant  |   |   |  |  |  |  |  |  |
|  | AR et al  |   |  |  |  |  |  |  |
| Amersham Biosciences AB et al  |   |   |  |  |  |  |  |  |
| This report is the international pre Authority under Article 35 and to         | liminary examination report, established by the ansmitted to the applicant according to Article           | is International Preliminary Examining<br>36.           |  |  |  |  |  |  |
| 2. This REPORT consists of a total of  | of 10 sheets, including this cover  | r sheet.  |  |  |  |  |  |  |
| 3. This report is also accompanied b   | y ANNEXES, comprising:  |   |  |  |  |  |  |  |
|  |   | sheets, as follows:                                     |  |  |  |  |  |  |
|  | and to the International Bureau) a total of   | e been amended and are the basis of this report         |  |  |  |  |  |  |
| and/or sheets  | containing rectifications authorized by this Average Instructions).                                       | othority (see Rule 70.16 and Section 607 of the         |  |  |  |  |  |  |
| sheets which   | supersede earlier sheets, but which this Autho  | rity considers contain an amendment that goes           |  |  |  |  |  |  |
| beyond the di<br>Supplementa   |   | ed, as indicated in item 4 of Box No. I and the         |  |  |  |  |  |  |
| b. (sent to the Internation  | onal Bureau only) a total of (indicate type and   | number of electronic carrier(s))                        |  |  |  |  |  |  |
|  | , containing a sequence listing   | and/or tables related thereto, in computer              |  |  |  |  |  |  |
| readable form only, a<br>Administrative Instru                                 | is indicated in the Supplemental Box Relating   | to Sequence Listing (see Section 802 of the             |  |  |  |  |  |  |
|  |   |   |  |  |  |  |  |  |
| 4. This report contains indications re  Box No. I Basis o                      | elating to the following items:  f the report   |   |  |  |  |  |  |  |
|  | •   |   |  |  |  |  |  |  |
| Box No. II Priority  |   | inventive step and industrial applicability             |  |  |  |  |  |  |
|  | tablishment of opinion with regard to novelty,  | myennive step and moustrial approaching                 |  |  |  |  |  |  |
| 1 1  | f unity of invention  |   |  |  |  |  |  |  |
| Box No. V Reason applica   | ned statement under Article 35(2) with regard to bility; citations and explanations supporting statements | to novelty, inventive step or industrial such statement |  |  |  |  |  |  |
| Box No. VI Certain   | documents cited   |   |  |  |  |  |  |  |
| Box No. VII Certair  | defects in the international application  |   |  |  |  |  |  |  |
| Box No. VIII Certain   | observations on the international application   |   |  |  |  |  |  |  |
| Date of submission of the demand   | Date of completio   | n of this report  |  |  |  |  |  |  |
| <b>Date of Gallering</b>   |   |   |  |  |  |  |  |  |
| 12.01.2004   | 03.08.200   | 03.08.2004  |  |  |  |  |  |  |
| Name and mailing address of the IPEA/S   |   |   |  |  |  |  |  |  |
| Patent- och registreringsverket  | 1   |   |  |  |  |  |  |  |
| Box 5055<br>S-102 42 STOCKHOLM   | Sara Nils   | son/Els   |  |  |  |  |  |  |
| Facsimile No. +46 8 667 72 88  | Telephone No. +   | 16 8 782 25 00  |  |  |  |  |  |  |
| Form PCT/IPEA/409 (cover sheet) (January 2004)                                 |   |   |  |  |  |  |  |  |



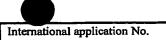


International application No.

PCT/SE 2003/001127

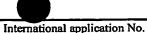
| Box | No. I   | Basis of the report  |  |  |  |  |  |
|-----|---|--|--|--|--|--|--|
| 1.  | With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item. |  |  |  |  |  |  |
|     |   | This report is based on a translation from the original language into the following language which is the language of a translation furnished for the purposes of:   |  |  |  |  |  |
|     |   | international search (under Rules 12.3 and 23.1(b))  |  |  |  |  |  |
|     |   | publication of the international application (under Rule 12.4)   |  |  |  |  |  |
|     |   | international preliminary examination (under Rules 55.2 and/or 55.3)   |  |  |  |  |  |
| 2.  | furnish   | th regard to the elements of the international application, this report is based on (replacement sheets which have been aished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" are not annexed to this report): |  |  |  |  |  |
|     | $\boxtimes$   | the international application as originally filed/furnished  |  |  |  |  |  |
|     |   | the description:   |  |  |  |  |  |
|     |   | pages as originally filed/furnished  |  |  |  |  |  |
|     |   | pages* received by this Authority on   |  |  |  |  |  |
|     |   | pages* received by this Authority on   |  |  |  |  |  |
|     |   | the claims:  |  |  |  |  |  |
|     |   | pages as originally filed/furnished  pages* as amended (together with any statement) under Article 19  |  |  |  |  |  |
|     |   |  |  |  |  |  |  |
|     |   | pages* received by this Authority on received by this Authority on   |  |  |  |  |  |
|     |   |  |  |  |  |  |  |
|     | ш   | the drawings:  as originally filed/furnished   |  |  |  |  |  |
|     |   | pages as originally intertainment pages* received by this Authority on   |  |  |  |  |  |
|     |   | pages* received by this Authority on   |  |  |  |  |  |
|     |   | a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.  |  |  |  |  |  |
| 3.  |   | The amendments have resulted in the cancellation of:   |  |  |  |  |  |
|     |   | the description, pages   |  |  |  |  |  |
|     |   | the claims, Nos.   |  |  |  |  |  |
|     |   | the drawings, sheets/figs  |  |  |  |  |  |
|     |   | the sequence listing (specify):  |  |  |  |  |  |
|     |   | any table(s) related to the sequence listing (specify):  |  |  |  |  |  |
| 4.  |   | This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).                                      |  |  |  |  |  |
|     |   | the description, pages   |  |  |  |  |  |
|     |   | the claims, Nos.   |  |  |  |  |  |
|     |   | the drawings, sheets/figs  |  |  |  |  |  |
|     |   | the sequence listing (specify):  |  |  |  |  |  |
|     |   | any table(s) related to the sequence listing (specify):  |  |  |  |  |  |
|     | * If item 4 applies, some or all of those sheets may be marked "superseded."  |  |  |  |  |  |  |
|     | ij nen  | is a upprice, some or an of more encousance, or manifest out - series.   |  |  |  |  |  |





PCT/SE 2003/001127

| Box No. II  | II Non-establishment of opinion with regard to novelty, inventive step and industrial applicability   |  |  |  |  |  |  |
|---|---|--|--|--|--|--|--|
| The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of: |   |  |  |  |  |  |  |
| ti  | the entire international application  |  |  |  |  |  |  |
|   | claims Nos.   |  |  |  |  |  |  |
| because:  |   |  |  |  |  |  |  |
| i t   | the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify): |  |  |  |  |  |  |
|   |   |  |  |  |  |  |  |
|   |   |  |  |  |  |  |  |
|   | ·   |  |  |  |  |  |  |
|   |   |  |  |  |  |  |  |
|   |   |  |  |  |  |  |  |
| L t   | the description, claims or drawings (indicate particular elements below) or said claims Nosare so unclear that no meaningful opinion could be formed (specify ):              |  |  |  |  |  |  |
|   |   |  |  |  |  |  |  |
|   |   |  |  |  |  |  |  |
|   |   |  |  |  |  |  |  |
|   |   |  |  |  |  |  |  |
|   |   |  |  |  |  |  |  |
|   | the claims, or said claims Nos are so inadequately supported by the description that no meaningful opinion could be formed.   |  |  |  |  |  |  |
|   | no international search report has been established for said claims Nos. 1-8, 10-17 all partially   |  |  |  |  |  |  |
|   | the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:                       |  |  |  |  |  |  |
| 1   | the written form has not been furnished   |  |  |  |  |  |  |
|   | does not comply with the standard   |  |  |  |  |  |  |
| 1   | the computer readable form has not been furnished   |  |  |  |  |  |  |
| _ <del></del> ,   | does not comply with the standard the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with              |  |  |  |  |  |  |
|   | the technical requirements provided for in the Annex C-bis of the Administrative Instructions.  |  |  |  |  |  |  |
|   | See Supplemental Box for further details.   |  |  |  |  |  |  |
|   |   |  |  |  |  |  |  |
| 1   |   |  |  |  |  |  |  |



PCT/SE 2003/001127

| Box No. V  |           | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |         |          |     |  |  |  |
|--|-----------|---|---------|----------|-----|--|--|--|
| 1.   | Statement |   |         |          | •   |  |  |  |
|  | Novel     | ty (N)  | Claims  | 1-17     | YES |  |  |  |
|  | 3.3.      |   | Claims  |          | NO  |  |  |  |
|  | Invent    | tive step (IS)  | Claims  | 16       | YES |  |  |  |
|  |           | - ' '   | Claims  | 1-15, 17 | NO  |  |  |  |
|  | Indust    | trial applicability (IA)  | Claims  | 1-17     | YES |  |  |  |
|  |           |   | Claims  |          | NO  |  |  |  |
|  |           |   | <u></u> |          |     |  |  |  |
| 2. Citations and explanations (Rule 70.7)        |           |   |         |          |     |  |  |  |
| The following documents are considered relevant: |           |   |         |          |     |  |  |  |

- - D1) US4055469
  - D2) EP1031626 A1
  - D3) Izumrudov V.A. et al, "Controllable stability of DNAcontaining polyelectrolyte complexes in water-salt solutions", Biopolymers (nucleic acid sciences), vol. 52, 94-108 (1999)
  - D4) Kabanov A. V. et al, "DNA interpolyelectrolyte complexes as a tool for efficient cell transformation, Biopolymers, vol. 31, 1437-1443 (1991)
  - Zelikin A. N. And Izumrudov V. A. "Polyelectrolyte D5) complexes formed by calf thymus DNA and aliphatic ionenes: unexpected change in stability upon variation of chain length of ionenes of different charge density", Macromol. Biosc. 2002, 2, 78-81
  - D6) EP0281390 A2
  - D7) US2002010145 A1
  - D8) Ramsden D. K. et al, "Flocculation of cellular material in flocculant medium with the fermentation complex poly(diallyldimethylammonium chloride)", Biotechnology techniques, vol. 12, no. 8, 1998
  - D9) US 5010183 A
  - accession number PREV19939610753 "Efficient D10) BIOSIS, separation of natural riobonucleotides by low-pressure anionexchange chromatography"
  - D1 shows a method for precipitation of nucleic acids. The method can be used to selectively precipitate nucleic acids from a solution containing proteins. Cationic polymers, e.g. polymers containing quaternary amines are used in the method disclosed. The binding of the polymers to the nucleic acid,



#### Supplemental Box

In case the space in any of the preceding boxes is not sufficient. Continuation of: Box  $\,V\,$ 

the effectiveness of complex formation and precipitation, is not strongly influenced by the pH. To determine the quantity of polymer to be added the quantity of nucleic acids present in the extract can be determined. The effects of charge precipitation polymer size on the and The effect of solutions with different salt investigated. concentration is investigated. Nucleic acids are precipitated leaving other species in the solution. The precipitation is performed on cell lysates. See especially col. 4 lines 39-51, col. 5 lines 36-41, col. 7 lines 30-34, col. 8 lines 13-15 and col. 9 lines 24-32.

D2 shows the isolation of RNA and/or genomic DNA using cationic ammonium salts containing 1-24 repeating units. Nucleic acids are isolated from HeLa cells. The nucleic acid can be separated from the precipitation complex and isolated. See abstract, p. 4 line 18-p. 5 line 26 and p.38 claim 28.

poly(N',N'e.g. binding between DNA and D3 the In dimethyldiallylammonium) chloride, ionene bromide or poly(N-The stability of the alkyl-4-vinylpyridinium) is studied. complexes at different salt concentrations is studied. By using a fluorescence spectroscopic assay, the formation of polyelectrolyte complex (PEC) is monitored and the charge ratio when the PEC is formed can be determined (the decrease in fluorescence seen when the charge ratio is about 1 or above the salt concentration). method Α depending on the destruction of DNA-containing PECs in watermonitoring salt solutions is also disclosed. It is stated that PECs formed by polycations with quaternary amine groups are pH independent and the least tolerant to destruction of added salt. A mentioned application is delivery of DNA to cells. See p. 97 right col. Paragraph 3, p. 98 right col. and figure 1, p. 99 figure 3, p. 103 and p.105.

D4 relates to methods for increasing DNA hydrophobicity via inclusion into an interpolyelectrolyte complex with polycations. E.g. poly(N-ethyl-4-vinylpyridinium)bromide is used. Conditions under which self-assembly of DNA and polycation occurs, formation of an interpolyelectrolyte

International application No.

PCT/SE 2003/001127

#### Supplemental Box

In case the space in any of the preceding boxes is not sufficient. Continuation of:  $Box\ V$ 

complex (IPC), are established. It is shown that the formation of a soluble IPC occurs at a molar ratio of polycation repeating units and nucleic acid groups between 0 and 0,5. Parallel with the soluble IPC an insoluble complex with higher

polycation content is formed. A plasmid is incorporated into an IPC and transformed into cells. See p. 1439 left column.

D5 relates to polyelectrolyte complexes between DNA and aliphatic ionenes. It is stated that the degree of polymerisation and charge density of the ionenes control the stability of the complexes, which might be crucial for applications such as bioseparation. See p. 81 right col. paragraph 2.

D6 shows the use of polycationic solid supports in the purification of nucleic acids form solutions containing contaminants. The cations can be quaternary amines. The bound nucleic acids can be recovered from the support. See p. 6 lines 52-61, p. 7 line 85- p. 8 line 7, p. 17 example 15.

D7 shows a method for selective precipitation of DNA or plasmid DNA by the addition of a compaction agent such as spermidine or spermine. It is stated that the method can be performed on cell lysates. See abstract and fig. 1.

D8 shows the use of poly(diallyldimethylammonium chloride) for flocculation of cellular material. The charge density of the polymer used is 100.

D9 shows a method for purifying DNA or RNA from a mixture of biological materials, which comprises adding a cationic detergent to a mixture. The biological material mixture may be intact cells or cell lysates. The cationic detergent can be a such cationic detergent quaternary amine alkylbenzyldimethylammonium salt. The detergent is added in an dissolve solubilize sufficient cells, to contaminating proteins and lipids in the mixture, and form insoluble hydrophobic complex between the nucleic acid and the detergent. The complex which comprises the RNA or DNA with the detergent is separated from the solubilized contaminants, and may be dissolved or dispersed in a polar organic solvent. Thereafter the DNA or RNA is recovered by the addition of a



International application No.

PCT/SE 2003/001127

#### Supplemental Box

In case the space in any of the preceding boxes is not sufficient. Continuation of:  $Box\ V$ 

salt, which promotes the dissociation of the complex. See col. 2 lines 48-52 and col. 3 lines 24-36.

D10 shows the use of anion exchangers containing quaternary ammonium functionalities for separating riobonucleotides.

The present application relates to the problem of selectively precipitating a nucleic acid from a solution containing other species while leaving said other species in the solution. This is achieved by using a polycationic precipitating agent being a highly charged linear polymer that comprises quaternary amino groups. The method allows precipitation within a broad window of pH values and salt concentrations and is not sensitive to addition of an excess of precipitating agent.

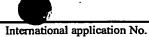
Document D1 is considered to represent the closest prior art.

The difference between the invention according to claim 1 and D1 is that the amount of precipitation agent (such an amount polycationic [+]/[-] between charge ratio about agent and nucleic acid is ≥ precipitating preferably ≥ about 1) used in claim 1 is not is not specified in D1. In D1, the amount of polymer to be added is not determined on the basis of the charge ratio.

The expression "about 1" and "about 0,5" used in claim 1 makes the scope of the claim unclear (see. PCT Art. 6). It is not clear what "about 1" or "about 0,5" means. The optimal charge ratio for forming a specific complex depends upon the salt concentration, but the vague expressions "about 1" and "about 0,5" nevertheless make the scope of the claim unclear.

By adding precipitating agent in the amounts mentioned above, it seems that an insoluble precipitation complex is attained. The precipitation complex is attained within a broad window of pH values and salt concentrations and it is not sensitive to addition of an excess of precipitating agent.

Consequently, with the background of D1, the problem is to attain an insoluble precipitation complex and thus an efficient precipitation in relation to the aspects mentioned



PCT/SE 2003/001127

#### Supplemental Box

In case the space in any of the preceding boxes is not sufficient. Continuation of: Box V

above, when performing a precipitation of a nucleic acid using a highly charged linear polymer.

The skilled person faced with the problem mentioned above, finds the solution in D3, which discloses the theory of the stability of polyelectrolyte complexes depending on the charge ratio and the amount of polymer added. D3 shows that the

polymers poly(N',N'-dimethyldiallylammonium) chloride, ionene bromide and poly(N-alkyl-4-vinylpyridinium) bind to DNA and that the stability of the complexes can be controlled by varying e.g. the salt concentration. The skilled person would consider D3 since the document relate to the binding of DNA to polycations, as do D1. The technical application in D3 differs from the application in D1-D2 but the skilled person would combine the documents since they share the same skilled person the obvious to Ιt is bioseparation. polyelectrolyte complexes can be used in Consequently, the invention according to claims 1-3, 5-12 and 17 is considered not to involve an inventive step given what is known form D1 in combination with D3. The addition of salt to dissolve or destruct the complex is investigated in D3. Consequently, the invention according to claims 11-15 considered not to involve an inventive step given what is known from D1 in combination with D3.

The same argumentation as made above can be made starting with D2 or D8-D9 as the document representing the closest prior art. It can be mentioned that D2 and D9 show the recovery of the nucleic acids after separating the precipitate.

In present claim 1 the expression "which method comprises to selectively precipitate the desired nucleic acid, leaving other species in solution" is used. By using this expression, the method is defined by reference to a result to not by technical method, the achieved by characterising how the method is performed. This way of defining the method leads to a lack of clarity (see PCT Art 6).

D1-D2 and D8-D9 show the precipitation of nucleic acids without the use of a strong base. Therefore, it is considered obvious to the skilled person that these methods can be used





International application No.

PCT/SE 2003/001127

### Supplemental Box

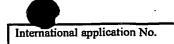
In case the space in any of the preceding boxes is not sufficient. Continuation of: Box  $\,V\,$ 

to precipitate different kinds of nucleic acids. Consequently, the invention according to claim 4 is considered not to involve an inventive step.

Nothing is mentioned in either documents D1-D1 or D8-D9 about isolating more than one desired nucleic acid by continued addition of precipitating agent. Therefore, the invention according to claim 16 is not considered obvious to the skilled person in view of the sited documents.

Documents D4-D7 and D10 are considered to represent the general state of the art.





PCT/SE 2003/001127

#### Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The expression "about 1" and "about 0,5" used in claim 1 makes the scope of the claim unclear (see. PCT Art. 6). It is not clear what "about 1" or "about 0,5" means. The optimal charge ratio for forming a specific complex depends upon the salt concentration, but the vague expressions "about 1" and "about 0,5" nevertheless make the scope of the claim unclear.

In present claim 1 the expression "which method comprises to selectively precipitate the desired nucleic acid, while leaving other species in solution" is used. By using this expression, the method is defined by reference to a result to be achieved by the method, not by technical features characterising how the method is performed. This way of defining the method leads to a lack of clarity (see PCT Art 6).